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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Alberto L. Mendoza

Serial No.: 09/082,232 Group Art Unit 1645

Filed : 1998 May 20

For : METHOD AND VACCINE FOR TREATMENT OF PYTHIOSIS INSIDIOSI IN HUMANS AND LOWER ANIMALS

Examiner : N. Minnifield

Assistant Commissioner for Patents

Washington, D. C. 20231

DECLARATION UNDER 37 CFR 1.132

Dear Sir:

The inventor, Alberto L. Mendoza, states as follows:

(1) Results were obtained in 30 new vaccinated equines with granulomatous lesions on different parts of their bodies and two humans from Thailand with infections caused by *P. insidiosum*. The diagnosis of pythiosis was confirmed in all equines and the two humans by serology. An immunodiffusion test and an ELISA specific to *P. insidiosum* infections were used (Mendoza, L., et al., Clin. Diagnost. Lab. Immuno. 4:715-718 (1997); and Mendoza, L., et al., J. Clin. Microbiol. 13: 813-816 (1986)). In some cases the diagnosis of equine pythiosis were also confirmed by

histopathology and in a few instances by culture. In the two human cases histopathology, culture, and cytokines profile were performed.

(2) Data was obtained before and post vaccination from 20 horses. In the remaining 10 horses no data, during the vaccination trial, were available (the owners and/or veterinarian practitioners did not report the data). In these ten cases, I was informed that eight of the ten horses were cured, while two did not respond to vaccination.

(3) This study was conducted in coordination with Bio-Medical International, Austin, Texas. This company possesses a USDA permit for the experimental use of the PIV in horses. The study in the 20 horses was conducted from 1997 to 1999. The equines in this study were located in different areas of southern Texas. The vaccinated equines were of different breeding backgrounds. Their ages were between 8 months and 20 years old and the chronicity of the lesions was one month or less. In four cases, however, the lesions were observed for more than two months.

(4) All cases of equine pythiosis in the study of paragraph (3) were vaccinated in the middle of the horses's neck with 100 µl of the *Pythium insidiosum* vaccine (PIV) (2.0 mg/ml) as described in Examples 1 and 2 and as claimed in the above referenced application. The PIV was applied twice. The second vaccine always took place fourteen days after the first

dose. The rationale for the second vaccination were based on the fact that the PIV does not trigger a protective immune response but a curative immunity of short duration. Research has shown that the response after vaccination is linked to T-helper cells (Th1) subset which triggers a cellular immunity, mainly T cytotoxic lymphocytes (Dixon, D. M., et al., Med. Mycol. 36 (Suppl. 1): 57-67 (1998); and Thitithanyanont, A., et al., Clin. Infec. Dis. 27:1394-1400 (1998)).

(5) In 20 vaccinated equines of paragraph (3) (horses with collected data) a mild delay type hypersensitivity (DTH) was observed at the site of vaccination (around 8 to 20 mm in diameter). Itching and the DTH response at the site of vaccination were the only two observed side effects. The DTH reaction disappeared seven days after vaccination. No other side effects were observed. Eighteen (18) equines cases were cured after vaccination. Two (2) chronic cases responded at first and then lesions reappeared. After this, vaccination did not work any more.

(6) The overall rate of cure in this new trial in horses was 87%, including the horses of paragraphs (2) and (3).

(7) In 1992, Mendoza et al (Mendoza, L., et al., Mycopathologia 119:89-95 (1992)) found that horses with pythiosis responded to the vaccine described therein according to the duration of their lesions. In

that study all acute cases (100%) were cured by the vaccine described therein, while none of the chronic cases (2 months or more) responded to vaccination. In that study, most equine cases had pythiosis for a month or less (acute cases). With the PIV of the present invention fifty percent (50%) of the chronic cases were cured. This property was absent in the earlier vaccines of the prior art.

(8) In addition to the Thai boy cured by the PIV described in the application, two new cases were treated in Thailand. One of them is currently being treated, the other responded very well to the PIV. These new trials in humans suggest that the vaccine is a good choice to treat human pythiosis when all the surgical and chemotherapeutic options have failed. The PIV generated minor side effects at the vaccination site (itching and a DTH response) in both humans and equines. This new trial confirmed previous reports of the minor side effects and safety of the PIV in humans and animals.

(9) In previous studies in equines and three humans from Thailand with the disease, the appearance of a DTH response at the site of vaccination was indicative of cure in the vaccinated humans and animals (Dixon, D. M., et al., Med. Mycol. 36 (Suppl. 1): 57-67 (1998); Mendoza, L., et al., Mycopathologia 119:89-95 (1992); and Thitithanyanont, A., et al., Clin. Infec. Dis. 27:1394-1400 (1998). This study confirmed those

reports.

(10) The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of the Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Albert L. Mendoza
Date: 07/28/99